THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 9, pp. 7619–7628, March 4, 2011

N-cadherin Regulates p38 MAPK Signaling via Association with JNK-associated Leucine Zipper Protein

IMPLICATIONS FOR NEURODEGENERATION IN ALZHEIMER DISEASE[S]

Received for publication, June 26, 2010, and in revised form, December 21, 2010 Published, JBC Papers in Press, December 22, 2010, DOI 10.1074/jbc.M110.158477

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Synaptic loss, which strongly correlates with the decline of cognitive function, is one of the pathological hallmarks of Alzheimer disease. N-cadherin is a cell adhesion molecule essential for synaptic contact and is involved in the intracellular signaling pathway at the synapse. Here we report that the functional disruption of N-cadherin-mediated cell contact activated p38 MAPK in murine primary neurons, followed by neuronal death. We further observed that treatment with Aβ_{42} decreased cellular N-cadherin expression through NMDA receptors accompanied by increased phosphorylation of both p38 MAPK and Tau in murine primary neurons. Moreover, expression levels of phosphorylated p38 MAPK were negatively correlated with that of N-cadherin in human brains. Proteomic analysis of human brains identified a novel interaction between N-cadherin and JNK-associated leucine zipper protein (JLP), a scaffolding protein involved in the p38 MAPK signaling pathway. We demonstrated that N-cadherin expression had an inhibitory effect on JLP-mediated p38 MAPK signal activation by decreasing the interaction between JLP and p38 MAPK in COS7 cells. Also, this study demonstrated a novel physical and functional association between N-cadherin and p38 MAPK and suggested neuroprotective roles of cadherin-based synaptic contact. The dissociation of N-cadherin-mediated synaptic contact by Aβ may underlie the pathological basis of neurodegeneration such as neuronal death, synaptic loss, and Tau phosphorylation in Alzheimer disease brain.

Alzheimer disease (AD)[2] is pathologically characterized by the presence of amyloid β-peptide (Aβ) and neurofibrillary tangles in the neocortex and hippocampus. Insoluble Aβ fibrillar aggregates found in senile plaques have long been considered to cause the neurodegeneration of AD. On the other hand, synaptic loss is another pathological hallmark of AD, which strongly correlates with the severity of cognitive impairment better than senile plaques or neurofibrillary tangles (1). Interestingly, recent studies from AD mouse models have shown that learning impairment and synaptic dysfunction become apparent before the formation of plaques, suggesting the hypothesis that soluble Aβ causes "synaptic failure" before plaques develop and neuron death occurs (2). Converging lines of evidence suggest that natural soluble Aβ oligomers trigger synaptic loss (3). Thus, in addition to the investigation of molecular mechanisms, which develop senile plaques and neurofibrillary tangles, research focusing on synaptic dysfunction is important to clarify the earliest pathology in AD.

Presenilin (PS) 1/2 is the essential catalytic component of γ-secretase proteolytic complex (4, 5), which is responsible for the final cleavage of amyloid precursor protein to generate Aβ peptides. Mutations in PS1 have been known as the most common cause of autosomal dominant familial Alzheimer disease (6–8). Interestingly, PS1 binds to N-cadherin, which is an essential molecule for synaptic contact and is abundantly localized in hippocampal synapses (9). The cytoplasmic domain of cadherin associates with the actin cytoskeleton via β-catenin and regulates synaptic contact, synaptogenesis, and dendritic spine morphology (10, 11). In addition to the structural role as an adhesive molecule, N-cadherin plays important roles in intracellular signaling pathways such as β-catenin or Wnt signaling. Also, N-cadherin-based cell-cell adhesion activates PI3K/Akt cell survival signaling by recruiting PI3K into the N-cadherin adhesion complex (12). Further, PS1 facilitates this process by promoting cadherin/PI3K association (13). As a consequence, PS1/N-cadherin interaction at the synapse seems to be neuroprotective by facilitating the PI3K/Akt survival signaling. Recently, we demonstrated that N-cadherin promotes the cell surface expression of PS1/γ-secretase, thereby activating the PI3K/Akt/GSK3β signaling pathway (14) and that N-cadherin-mediated synaptic adhesion modulates Aβ secretion as well as A$\beta_{42/40}$ ratio via PS1/

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S5.

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[2] The abbreviations used are: AD, Alzheimer disease; Aβ, amyloid β; JLP, JNK-associated leucine zipper protein; PS, presenilin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ANOVA, analysis of variance.

N-cadherin interactions (15). Therefore, it is plausible that N-cadherin functions not only as a synaptic adhesion molecule but also as a modulator of AD pathology by affecting Aβ production and PI3K/Akt signaling.

On the other hand, increased p38 MAPK activity is associated with the neuropathology of AD (16). For example, both p38 MAPK and its upstream kinase MKK6 are activated in AD brain tissue as demonstrated by immunohistochemistry (17, 18). Activation of the p38 MAPK signaling is also reported in an AD-relevant animal model (19). Moreover, a previous report demonstrated that Aβ employs synaptic depression to drive endocytosis of synaptic AMPA receptor by activating p38 MAPK (20). Among the MAPK family members, p38 MAPK is activated by numerous unique signals such as environmental stressors and toxins, cellular injury, growth factors, and inflammatory cytokine leading to various neuronal cell fates including apoptosis, differentiation, and proliferation. However, the relationship between p38 MAPK and N-cadherin has not been elucidated in AD pathology.

Based on the above observations, we hypothesized that disruption of N-cadherin-based cell-cell contact may up-regulate the p38 MAPK signaling, leading to the neurodegeneration of AD. We demonstrated an inverse correlation between the expression levels of phosphorylated (and thus activated) p38 MAPK and those of N-cadherin in human brains. Moreover, we showed that the disruption of N-cadherin-based contact leads to an activation of p38 MAPK signaling in murine primary neurons, followed by neuronal death. Furthermore, we performed proteomic analysis using human brains and identified JNK-associated leucine zipper protein (JLP) as a novel interacting protein of N-cadherin, thus demonstrating a new signaling pathway from N-cadherin to p38 MAPK through the association with JLP, which might be compromised in AD pathogenesis.

EXPERIMENTAL PROCEDURES

Human Material and Proteomics—Human brain tissues were provided by the Tokyo Institute of Psychiatry. Brains from non-AD and AD patients were dissolved in radioimmune precipitation assay buffer (50 mM Tris, pH 8.0, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 1% Nonidet P-40, and 0.5% deoxycholate) supplemented with protease inhibitor mixture (Roche Applied Science) and phosphatase inhibitor mixture (Sigma). Each sample was then centrifuged at 14,000 $\times$ *g* for 20 min at 4 °C, and the supernatants were collected to obtain soluble proteins. Protein concentration was determined using the Bradford assay. Equal amounts of protein were subjected to SDS-PAGE followed by Western blot. For proteomic analysis, equal amounts of aliquots were treated with protein G-Sepharose (GE Healthcare) for 1 h at 4 °C. After removing protein G-Sepharose by centrifugation at 2,000 $\times$ *g* for 5 min, anti-N-cadherin antibody (BD Biosciences) was added to the supernatants. Each sample was rotated for 2 h at 4 °C and then treated with protein G-Sepharose for 1 h at 4 °C. The immunoprecipitates were washed with radioimmune precipitation assay buffer five times and resuspended in 2$\times$ sample buffer (125 mM Tris-HCl, pH 6.8, 4.3% SDS, 30% glycerol, 10% 2-mercaptoethanol, and 0.01% bromphenol blue). After boiling for 4 min, the supernatants were subjected to SDS-PAGE. To visualize proteins, the gels were stained with silver nitrate using PlusOne silver staining kit protein (GE Healthcare). The protein bands were excised and subjected to in gel trypsinization, and molecular mass analysis of the tryptic peptides was performed by MALDI-TOF/MS with an Ultraflex MALDI-TOF/TOF system (Bruker Daltonics, Billerica, MA).

Cells, Plasmids, and Transfection—HEK293 and COS7 cells were maintained in DMEM (Sigma) containing 10% FBS (Invitrogen) and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 incubator. SH-SY5Y cells, which are derived from human neuroblastoma cell lines, were maintained in Opti-MEM® (Invitrogen) containing 10% FBS. Primary neurons were obtained from the cerebral cortices of fetal mice (14–16 days of gestation) and cultured in neurobasal medium supplemented with B-27 (Invitrogen). Expression plasmids encoding S-tagged JLP and its mutant derivatives were kind gifts from Dr. Reddy (Temple University) (21). FLAG-tagged p38 MAPK and FLAG-tagged MKK4 (SEK1) were described previously (22). HA-tagged MEKK3 (Addgene plasmid 12186) was provided by Dr. Johnson (National Jewish Center for Immunology and Respiratory Medicine) (23). HA-tagged N-cadherin was described elsewhere (14). Transfection of either HEK293 or COS7 cells was carried out using Transfectin reagent (Bio-Rad) according to the manufacturer's protocol.

Antibodies and Reagents—The following antibodies were used in the study: mouse monoclonal antibody to N-cadherin (BD Biosciences), rabbit polyclonal antibody to JLP (Abcam), rabbit polyclonal antibody to p38 and phospho-p38 (Cell Signaling Technology), rabbit polyclonal antibody to S-probe (Santa Cruz Biotechnology), monoclonal and rabbit polyclonal anti-HA antibodies, mouse monoclonal anti-N-cadherin N terminus antibody (N-cadherin neutralizing antibody, GC-4), anti-β-actin antibody, anti-FLAG-M2 antibody, control normal mouse IgG (Sigma), mouse monoclonal antibody to PHF-Tau (AT8) (Pierce), and Alexa Fluor 546 goat anti-rabbit IgG conjugate and Alexa Fluor 488 goat anti-mouse IgG conjugate (Molecular Probe). ADH-1 was a kind gift from Dr. Gupta (Adherex Technologies Inc.). Synthetic Aβ_{42} peptides were obtained from Peptide Institute Inc. SB203580 was purchased from Calbiochem. S-protein-agarose beads were from Novagen.

Western Blot, Immunoprecipitation, Pulldown Assay, MTT Assay, and Cell Treatment by Reagents—Preparation of protein samples, Western blot, and immunoprecipitation were carried out as described elsewhere (14). Pull-down assay using S-protein-agarose beads (Novagen) was carried out as described elsewhere (21). MTT assay was performed using the MTT cell proliferation assay kit (Cayman) according to the manufacturer's instructions. For inhibition of N-cadherin-mediated cell-cell contact, the cells were treated either with ADH-1 as indicated or with N-cadherin-neutralizing antibody (GC-4) as described elsewhere (15).

Immunostaining—The samples for immunostaining were prepared as described elsewhere (15). After fixation, the samples were examined using a laser scanning confocal microscope, LSM 510 META (Zeiss).

TABLE 1
Characteristics of human cases
Clinical and histopathological information on brain samples used for analysis in Fig. 1*A*. We analyzed five AD patient brains confirmed by neuropathology and five control subjects without neurological complications. There is no statistical difference in age between AD and control cases. NA, not available. NFT, neurofibrillary tangle.

Case	Age	Sex	Post-mortem interval	Clinical diagnosis	Pathological findings
	years		*h*		
Non-AD					
Case 1	60	Male	NA	Alcoholism	Plaque(−), NFT stage I
Case 2	80	Female	NA	Abdominal aortic aneurysm rupture	Plaque(−), NFT stage II
Case 3	77	Male	7.5	Liver cirrhosis	Plaque(−), NFT stage II
Case 4	66	Male	11	Rectal cancer	Plaque(−), NFT stage I
Case 5	48	Male	10	Familial idiopathic basal ganglia calcification	Plaque(−), tangle(−)
AD					
Case 1	75	Male	12	Alzheimer disease	Braak stage C, VI
Case 2	68	Female	9	Alzheimer disease	Braak stage C, VI
Case 3	75	Male	17.5	Alzheimer disease	Braak stage C, VI
Case 4	81	Female	61	Dementia with Levy bodies	Alzheimer disease Braak stage C, VI
Case 5	56	Male	18	Alzheimer disease	Braak stage C, VI

Statistical Analysis—Signals on films were quantified with National Institutes of Health Image software (National Institutes of Health). Comparisons were performed using a Mann-Whitney *U* test or a Student's *t* test. For comparison of multiparametric analysis, one-way ANOVA, followed by the post hoc analysis by Fisher's protected least significant difference (PLSD) was used. Pearson's correlation co-efficients and significance were defined by STATVIEW software. The data are expressed as the means ± S.E., and statistical significance was assessed at $p < 0.05$.

RESULTS

The Expression Levels of Phosphorylated p38 MAPK Were Negatively Correlated with N-cadherin Expression Levels in Human Brains—N-cadherin is an essential adhesion molecule for forming synapses, and synaptic loss is one of pathological hallmarks of AD. Because previous reports showed that synaptic proteins such as synaptophysin or PSD-95 were reduced in AD (24, 25), we hypothesized that N-cadherin expression is also decreased in the brains of AD patients. First, the expression levels of N-cadherin were analyzed in human brain tissues from temporal cortices of AD patients and age-matched non-AD controls (Table 1). As expected, Western blot analysis using anti-N-cadherin antibody showed that expression levels of N-cadherin were decreased in AD brains compared with non-AD controls (Fig. 1*A*). Quantitative analysis showed that the ratio of N-cadherin/β-actin was significantly decreased in AD brains compared with that in non-AD controls (Fig. 1*B*, $p < 0.05$). To investigate whether the phosphorylation of p38 MAPK was enhanced in AD brains, we subsequently examined the expression levels of both phosphorylated and total p38 MAPK in the same tissues of human brains by Western blot (Fig. 1*A*). Consistent with previous reports (18–20), quantitative analysis showed a significant increase in the ratio of phospho/total p38 MAPK in AD brains, compared with that in non-AD controls (Fig. 1*C*, $p < 0.01$). Moreover, when we plotted the ratio of phospho/total p38 MAPK against that of N-cadherin/β-actin, we found that the phospho/total p38 MAPK ratio negatively correlated with N-cadherin/β-actin ratio (Fig. 1*D*, $r = -0.774$, $p < 0.01$). These results suggested a negative correlation between phosphorylated p38 MAPK and N-cadherin expressions in human brain.

ADH-1 (N-cadherin Antagonist) Induced Neuronal Cell Death by Activating p38 MAPK in Murine Primary Neurons—To elucidate the link between the reduced level of N-cadherin and p38 MAPK activation in AD brains, we analyzed whether the inhibition of N-cadherin-based synaptic contact could lead to p38 MAPK activation in neuronal cells. The N-terminal extracellular domain of N-cadherin harbors the homophilic cell adhesion recognition sequence, His-Ala-Val (HAV). It has been established that ADH-1, which mimics the natural HAV sequence of N-cadherin, can specifically disrupt N-cadherin-mediated cell adhesion (26). Therefore, we used it as a specific N-cadherin antagonist in the present experiment. First, murine primary neurons were treated with different concentrations of ADH-1 to disrupt the N-cadherin-based synaptic contact, followed by analysis of p38 MAPK activation by Western blot. Interestingly, exposure of murine primary neurons to ADH-1 for 24 h enhanced the phosphorylation of p38 MAPK and Tau in a concentration-dependent manner (Fig. 2*A* and supplemental Fig. S1). To confirm the effect of N-cadherin inhibition on p38 MAPK signaling and Tau phosphorylation in an alternative way, we applied N-cadherin-neutralizing antibody to murine primary neurons for 6 h followed by Western blot, and the same result was obtained as ADH-1 (supplemental Fig. S2). Next, we asked whether activation of p38 MAPK after inhibition of N-cadherin-based synaptic contact could lead to neuronal death. To answer this question, we examined neuronal cell viability after ADH-1 treatment using MTT assay. We observed significant decreases in neuronal cell viability after treatment with ADH-1 ($n = 4$, $p < 0.001$) (Fig. 2*B*). To evaluate whether p38 MAPK plays an important role in ADH-1-induced neuronal death, we used SB203580, a well characterized p38 MAPK-specific inhibitor. ADH-1 application with or without SB203580 revealed that neuronal death induced by ADH-1 was significantly attenuated by co-treatment with SB203580 ($n = 4$, $p < 0.001$) (Fig. 2*C*). Thus, the increased level of p38 MAPK activation is responsible for the ADH-1-induced neuronal death. To prove the effect of ADH-1 on the structural integrity of N-cadherin-mediated cell contact and p38 MAPK activation, ADH-1 (0.5 mg/ml) was added to confluent SH-SY5Y cells for 24 h. As expected, confocal microscopic analysis showed that

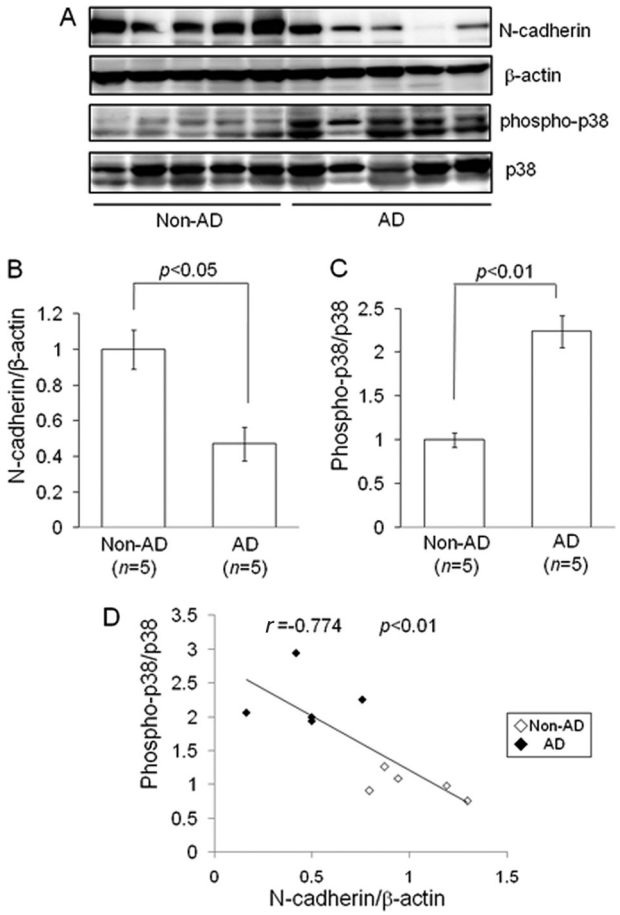

FIGURE 1. **Increased phospho-p38 MAPK expressions were negatively correlated with decreased N-cadherin expressions in human brains.** *A*, brain homogenates of temporal cortex from AD patients (*AD*, $n = 5$) and age-matched non-AD controls (*Non-AD*, $n = 5$) were analyzed by Western blot using anti-N-cadherin, β-actin, phospho-p38, and p38 MAPK antibodies. *B*, the band densities of N-cadherin and control β-actin were quantified by National Institutes of Health Image. The ratio of N-cadherin to β-actin (*N-cadherin/β-actin*) was calculated and analyzed by Mann-Whitney's *U* test. The N-cadherin/β-actin ratio was significantly decreased in the brains of AD patients compared with that of non-AD controls ($p < 0.05$). *C*, the band densities of phospho-p38 and p38 MAPK were quantified by National Institutes of Health Image. The phospho/total p38 MAPK ratio was calculated and analyzed by Mann-Whitney's *U* test. The phospho/total p38 MAPK ratio was significantly increased in the brains of AD patients compared with that of non-AD controls ($p < 0.01$). *D*, significant correlation was established in comparison between the phospho/total p38 MAPK ratio and the N-cadherin/β-actin ratio by Pearson's correlation co-efficients. The phospho/total p38 MAPK ratio was negatively correlated with the N-cadherin/β-actin ratio in human brain samples. ($r = -0.774$, $p < 0.01$).

the treatment with ADH-1 resulted in the significant decrease of N-cadherin immunoreactivity at the sites of cell-cell contact (Fig. 2*D*, *left panels*), indicating the disruption of N-cadherin-mediated cell contact. Consistent with the above result, enhanced immunoreactivity of phospho-p38 MAPK was observed in cells treated with ADH-1, compared with non-treated cells (Fig. 2*D*, *middle panels*). No immunoreactivities of both N-cadherin and phospho-p38 MAPK were observed in the absence of primary antibodies (supplemental Fig. S3).

Aβ$_{42}$ Decreased N-cadherin Expression through NMDA Receptors in Murine Primary Neurons—A number of recent studies have found that Aβ is synaptotoxic (20, 27). Therefore, we next examined whether Aβ could down-regulate N-cadherin expression in neurons. To assess the effect of Aβ on N-cadherin expressions, murine primary neurons were treated with synthetic Aβ$_{42}$ peptides (100 nM) for 48 h and subjected to Western blot. As shown in Fig. 3*A*, Aβ$_{42}$ treatment decreased N-cadherin expression in neurons. Previous reports have demonstrated that p38 MAPK is activated by the fibrillar Aβ (28) and that p38 MAPK can phosphorylate Tau at Ser-202/Thr-205 (29). Thus, we examined whether synthetic Aβ$_{42}$ peptides could trigger p38 MAPK activation and Tau phosphorylation at Ser-202/Thr-205 in our system. Consistent with the previous reports, we observed increased levels of phosphorylated p38 MAPK and phosphorylated Tau in the Aβ$_{42}$-treated cell preparations as compared with nontreated ones by Western blot ($n = 3$, $p < 0.01$) (Fig. 3, *A* and *B*). Together, this experiment indicated that Aβ$_{42}$ treatment decreases the levels of N-cadherin, activates p38 MAPK, and phosphorylates Tau in neuronal cells. Previous reports demonstrated that Aβ treatment induces excessive excitation of glutamate receptors to cause excitotoxicity (30, 31). Thus, we hypothesized that the decreased level of N-cadherin was induced by the Aβ-mediated excitotoxicity. To test this, murine primary neurons were pretreated (30 min) with the NMDA receptor antagonist MK-801 (10 μM) before being exposed to Aβ$_{42}$. Subsequent Western blot analysis of cell lysates revealed that MK-801 inhibited Aβ$_{42}$-induced reduction of N-cadherin levels ($n = 3$, $p < 0.05$) (Fig. 3*C*).

N-cadherin Associates with JLP Both in Human Brains and in Murine Primary Neurons—To clarify the mechanistic link between the disruption of N-cadherin-based contact and p38 MAPK activation, we set out to identify the proteins that associate with N-cadherin in human brain samples. For this, lysates of temporal cortices from brains of AD and non-AD patients were immunoprecipitated with anti-N-cadherin antibody, and the immunoprecipitates were subsequently subjected to SDS-PAGE. The separated proteins were visualized by silver staining, demonstrating a protein band of ~180 kDa in the lysates of brains of both AD patients and non-AD controls (supplemental Fig. S4). The protein band was identified by mass spectrometry as JLP, a scaffold protein that has been known to mediate the interaction between p38 MAPK and its upstream kinases (21). To confirm the association between N-cadherin and JLP, we transiently transfected HA-tagged N-cadherin and/or FLAG-tagged JLP expression constructs into HEK293 cells. Equal amounts of cell lysates obtained from each transfected cell were immunoprecipitated with anti-HA antibody and then immunoblotted with anti-FLAG antibody (Fig. 4*A*). A protein band of ~180 kDa was visualized with anti-FLAG antibody in the immunoprecipitate using anti-HA antibody obtained from the double-transfected cells (Fig. 4*A*, *third lane*), clearly indicating that N-cadherin associates with JLP. To further verify this association, the lysates derived from HEK293 cells transiently transfected with HA-tagged N-cadherin and/or FLAG-tagged JLP plasmids were immunoprecipitated with anti-FLAG antibody. As shown in Fig. 4*B*, HA-tagged immunoreactivity was observed in the immunoprecipitate with anti-FLAG antibody, confirming the association of N-cadherin with JLP. Finally, to demonstrate the endogenous association between N-cadherin and JLP in

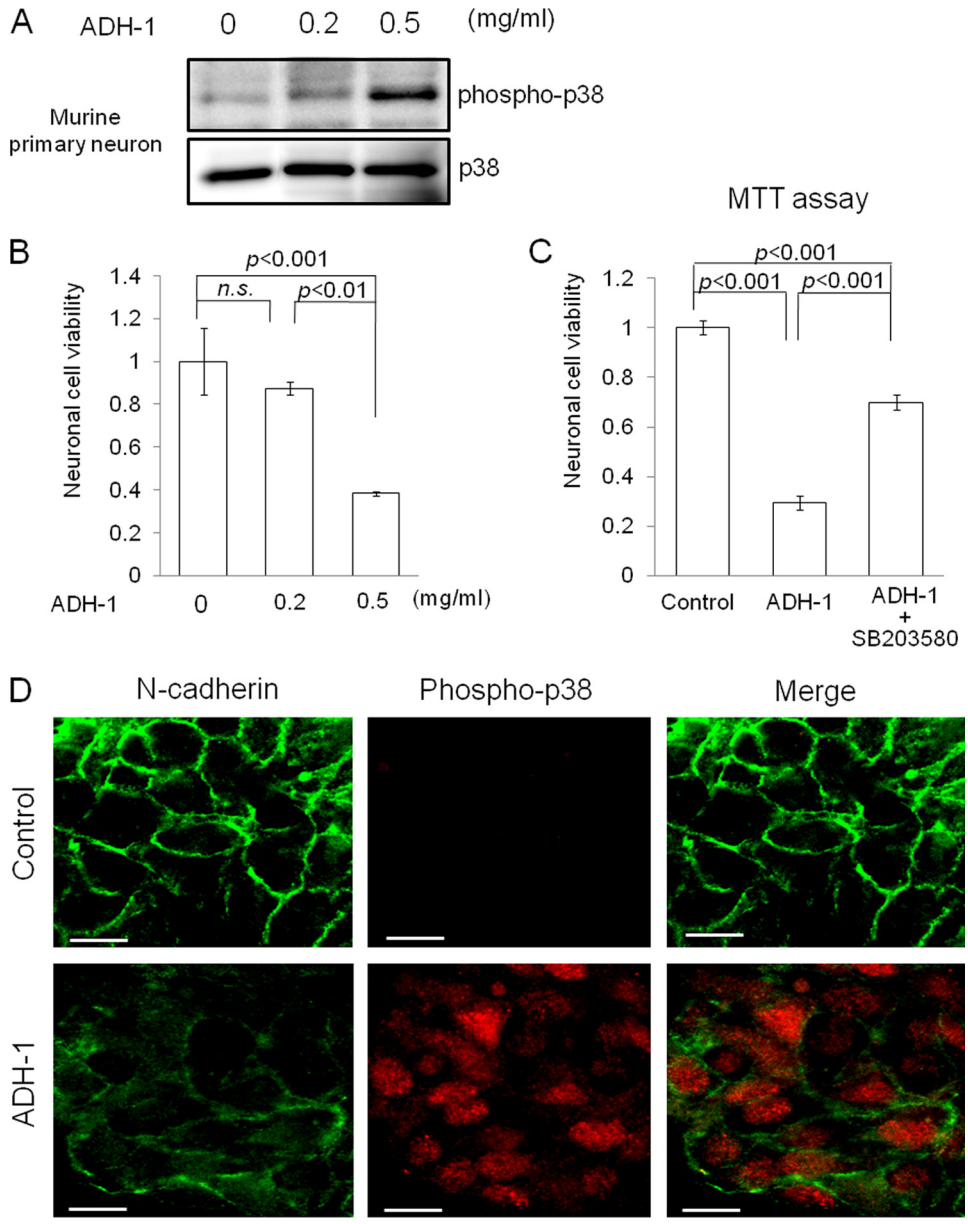

FIGURE 2. **ADH-1 induced neuronal cell death by activating p38 MAPK in murine primary neurons.** *A*, ADH-1, N-cadherin antagonist, was applied to murine primary neurons at different concentrations as shown. 24 h after treatment, the lysates were immunoblotted with anti-phospho-p38 and p38 MAPK antibodies. ADH-1 increased phosphorylation of p38 MAPK. *B*, neuronal cell death induced by ADH-1 was evaluated by MTT assay ($n = 5$, $p < 0.001$). *C*, murine primary neurons were treated with ADH-1 (0.5 mg/ml) for 24 h with or without 30 min of pretreatment with 10 μM SB203580, a specific p38 MAPK inhibitor, and the cell death was examined by MTT assay ($n = 4$, $p < 0.001$). SB203580 attenuated neuronal cell death induced by ADH-1 ($n = 4$, $p < 0.001$). *D*, ADH-1 (0.5 mg/ml) was added to confluent SH-SY5Y cells for 24 h followed by the immunostaining, using anti-N-cadherin and anti-phospho-p38 antibodies. Treatment with ADH-1 perturbed N-cadherin immunoreactivity, indicating a partial loss of N-cadherin from cell-cell junctions. *Scale bar*, 10 μm.

neurons, murine primary neurons were lysed, immunoprecipitated with anti-N-cadherin antibody, and then subjected to Western blot with anti-JLP antibody (Fig. 4*C*). The result showed endogenous association between N-cadherin and JLP in murine primary neurons, as well as in human brains.

N-cadherin/JLP Association Is Mediated by the Region Spanning Amino Acids 160–209 and Leucine Zipper II Domain of JLP—To determine the N-cadherin-binding domains of JLP, a series of S-tagged JLP mutants truncated at the C terminus was transfected into COS7 cells (Fig. 5*A*). Immunoprecipitation with anti-N-cadherin antibody showed that all of the S-tagged C-terminal deletion mutants of JLP were associated with N-cadherin (Fig. 5*B*), indicating that the JLP N terminus plays an important role in the association with N-cadherin. To further characterize the sequences of JLP involved in this association, various N-terminally truncated JLP mutants tagged with the S-sequence were prepared as indicated (Fig. 5*C*). COS7 cells were transiently transfected with these deletion mutants, and the lysate derived from each transfected cell was subjected to immunoprecipitation assay with anti-N-cadherin antibody. We observed that the JLP fragment corresponding to the region of amino acids 160-463 was strongly associated with N-cadherin, whereas that corresponding to the region of amino acids 160–398 showed a weaker association with N-cadherin (Fig. 5*D*). In contrast, a shorter fragment corresponding to the region of amino

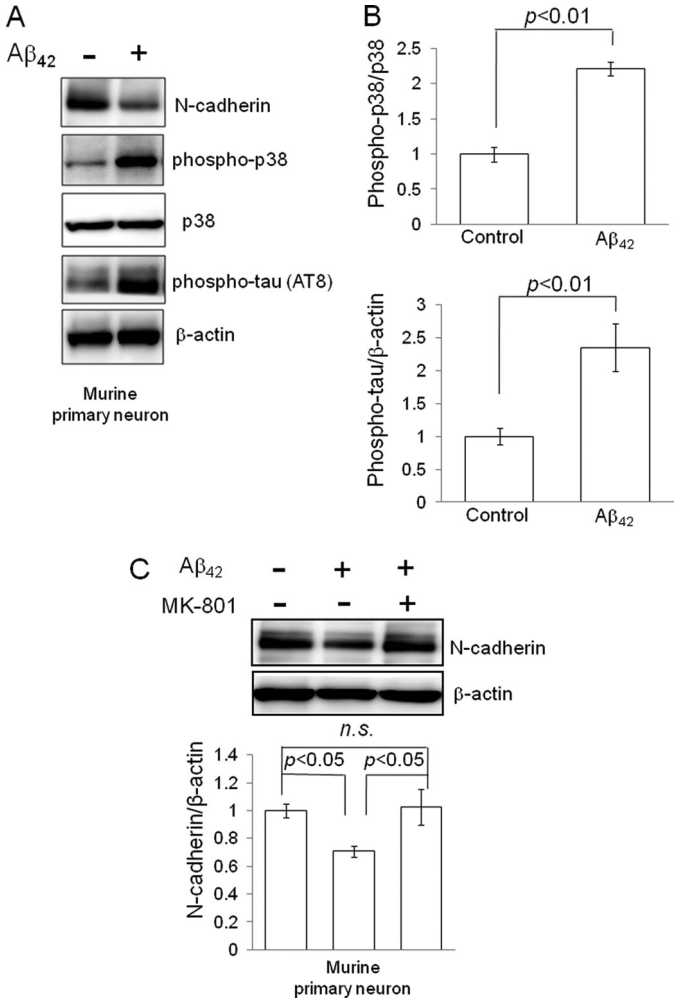

FIGURE 3. **Aβ_{42} decreased N-cadherin expressions through NMDA receptors in murine primary neurons.** *A*, murine primary neurons were treated with or without 100 nM synthetic Aβ_{42} peptides for 48 h, and the lysates were immunoblotted with anti-N-cadherin, phospho-p38, p38 MAPK, phospho-Tau (AT8), and β-actin antibodies, successively. Aβ_{42} treatment reduced N-cadherin expressions and induced phosphorylation of p38 MAPK and Tau in murine primary neurons. *B*, the band densities of phospho-p38, p38 MAPK, phospho-Tau (AT8), and β-actin were quantified by National Institutes of Health Image. The ratios of phospho/total p38 MAPK and phospho-Tau (AT8)/β-actin were calculated and analyzed by Student's *t* test ($n = 3$, $p < 0.01$). *C*, murine primary neurons were pretreated with 10 μM MK-801, NMDA receptor antagonist for 30 min followed by 100 nM synthetic Aβ_{42} peptides for 48 h. The lysates were evaluated by Western blot using anti-N-cadherin and β-actin antibodies. The band densities of N-cadherin and β-actin were quantified by National Institutes of Health Image. The N-cadherin/β-actin ratio was calculated and analyzed by one-way ANOVA. NMDA receptor antagonist MK-801 prevented the Aβ_{42}-induced decrease in N-cadherin levels ($n = 3$, $p < 0.05$).

acids 210–398, lacking amino acids 160–209 and leucine zipper II domain of amino acids 398–463, failed to co-immunoprecipitate with N-cadherin. These results implicated that both the region containing amino acids 160–209 and leucine zipper II domain in JLP are essential for its association with N-cadherin.

N-cadherin Expression Had a Negative Effect on p38 MAPK Activation by Inhibiting JLP/p38 MAPK Association—We next focused on investigating the functional role of the molecular association between N-cadherin and JLP. Previously, JLP has been reported to act as a scaffolding protein to bring p38 MAPK together with their upstream kinases MKK4 and MEKK3 (21), thereby activating the p38 MAPK pathway. As demonstrated above, we found that one of the N-cadherin-binding domains is the region spanning amino acids 160–209 of JLP. Interestingly, the same JLP domain has been identified to be involved in the association with p38 MAPK (21). Therefore, we hypothesized that N-cadherin could regulate the p38 MAPK signaling pathway via modulating JLP-p38 MAPK association. To prove this hypothesis, we investigated the effect of N-cadherin expression on the p38 MAPK signaling pathway in COS7 cells. FLAG-tagged p38 MAPK was transiently co-transfected into COS7 cells together with FLAG-tagged MKK4, S-tagged JLP, and HA-tagged N-cadherin as designated in Fig. 6*A*. The cell lysates were analyzed by immunoblotting with anti-phospho-p38 MAPK or p38 MAPK antibodies, respectively (Fig. 6*A*). Consistent with the previous report, the phosphorylation of p38 MAPK was enhanced by the co-expression of JLP and MKK4, upstream kinase for p38 MAPK ($n = 3$, $p < 0.05$). Interestingly, we found that this activation of p38 MAPK induced by the co-expression of MKK4 and JLP was significantly suppressed by the co-expression of N-cadherin ($n = 3$, $p < 0.05$). Alternatively, we co-transfected HA-tagged MEKK3, another upstream kinase of p38 MAPK, into COS7 cells. Similarly, we observed that the enhancement of phosphorylated p38 MAPK under the co-expression of both MEKK3 and JLP was significantly suppressed by the co-expression of N-cadherin (Fig. 6*B*, $n = 3$, $p < 0.05$). Moreover, when we pull down JLP with S-agarose, we found a reduced interaction between JLP and p38 MAPK in the presence of N-cadherin (Fig. 6), indicating that N-cadherin competitively inhibits the binding of p38 MAPK to JLP. Indeed, co-transfection of FLAG-tagged p38 MAPK, FLAG-tagged MKK4, and JLP fragment corresponding to the region of amino acids 160–463, which could associate with N-cadherin into COS7 cells, showed the increased phosphorylation of p38 MAPK (supplemental Fig. S5). This result implies that this short fragment of JLP may compete with the endogenous JLP for the N-cadherin binding, resulting in the release of JLP from the N-cadherin and subsequent p38 MAPK activation. Taken together, these results suggested that N-cadherin expression had an inhibitory effect on the p38 MAPK signaling pathway via inhibition of JLP-p38 MAPK association.

DISCUSSION

The molecular mechanism of synaptic adhesion and cell viability pathway of p38 MAPK is not well understood yet. Our present study suggested a potentially new signaling pathway contributing to the aberrant activation of p38 MAPK in AD. We showed that ADH-1 caused a significant increase in p38 MAPK activation and Tau phosphorylation with a subsequent decrease in neuronal viability (Fig. 2, *A* and *B*, and supplemental Fig. S1). In alternative ways, application of N-cadherin-neutralizing antibody also activated p38 MAPK and increased phosphorylation of Tau in murine primary neurons (supplemental Fig. S2), suggesting that the disruption of N-cadherin-based cell adhesion could lead to p38 MAPK activation and Tau phosphorylation. Importantly, we demonstrated that the inhibition of the p38 MAPK pathway with specific

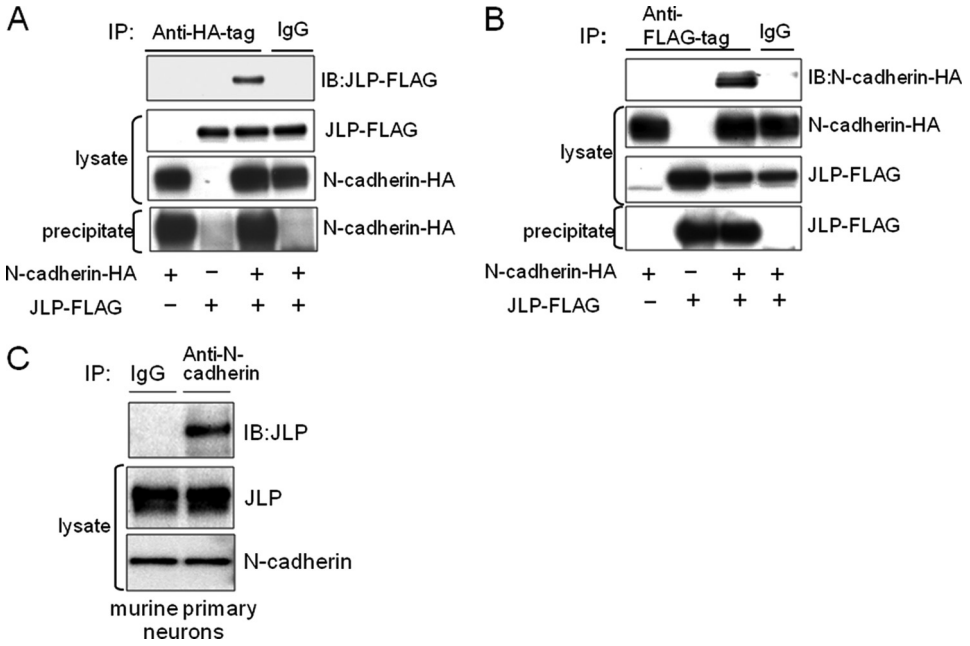

FIGURE 4. **The association of N-cadherin with JLP in HEK293 cells and neuronal cells.** *A*, lysates of HEK293 cells, transiently transfected with HA-tagged N-cadherin and/or FLAG-tagged JLP expressing vectors, were immunoprecipitated (*IP*) with anti-HA antibody (*lanes 1–3*) or normal IgG (*lane 4*). The immunoprecipitates and the lysates were analyzed by immunoblotting (*IB*) with the specific antibodies against the FLAG tag and HA tag. *B*, lysates of HEK293 cells, transiently transfected with HA-tagged N-cadherin and/or FLAG-tagged JLP expressing vectors were immunoprecipitated with anti-FLAG antibody (*lanes 1–3*) or normal IgG (*lane 4*). The immunoprecipitates and the lysates were analyzed by Western blot with the specific antibodies against the HA tag and FLAG tag. *C*, the endogenous association between N-cadherin and JLP in murine primary neurons was analyzed. The neurons were lysed and immunoprecipitated with either anti-N-cadherin antibody or normal IgG. The immunoprecipitates and the lysates were immunoblotted with the specific antibodies against JLP and N-cadherin.

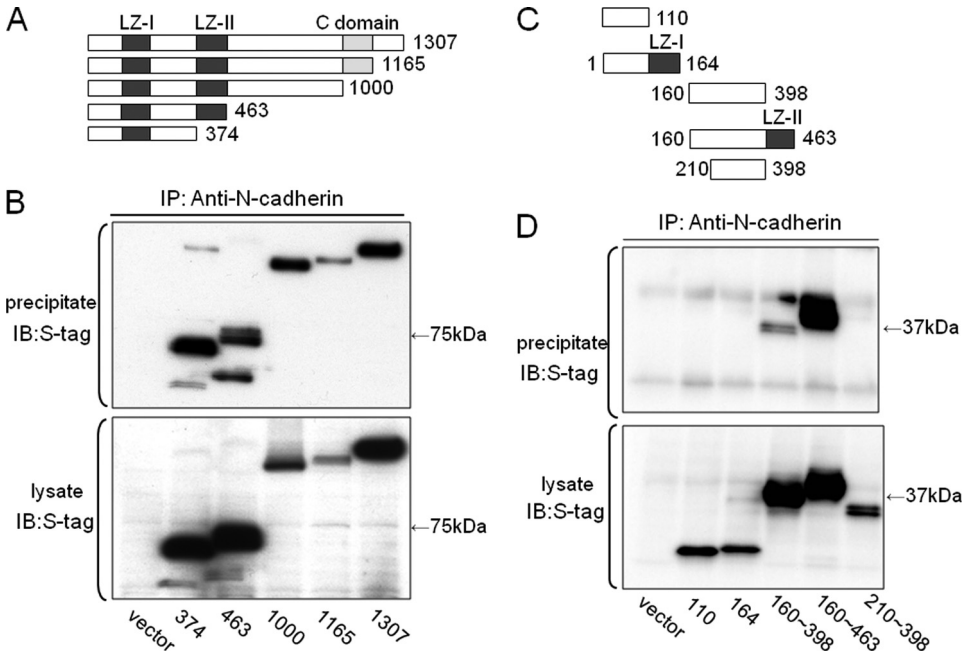

FIGURE 5. **Deletion mapping of N-cadherin-interacting domain of JLP.** *A*, schematic diagram of the C-terminally truncated S-tagged JLP constructs used for analysis of the JLP-N-cadherin interaction. The sizes of these deletion mutants were 374 (amino acids 1–374), 467 (amino acids 1–467), 1000 (amino acids 1–1000), 1165 (amino acids 1–1165), and 1307 amino acids (full length, residues 1–1307), respectively. *B*, COS7 cells were transiently transfected with the WT or C-terminally truncated mutants of S-tagged JLP. The lysates were immunoprecipitated (*IP*) with anti-N-cadherin antibody. The immunoprecipitates and the lysates were subjected to Western blot analysis with the specific antibodies against S-tag. All of these deletion mutants of JLP were co-immunoprecipitated with N-cadherin. *C*, schematic diagram representing shorter constructs of C-terminally truncated S-tagged JLP. N-terminally truncated short constructs were also prepared as shown in Fig. 5*C*. *D*, COS7 cells were transiently transfected with these different mutants of S-tagged JLP. The lysates were immunoprecipitated with anti-N-cadherin antibody, and the immunoprecipitates were immunoblotted (*IB*) with anti-S-tag antibody. The mutants consisting of amino acids 160–398 and 160–463 of JLP were co-immunoprecipitated with N-cadherin, whereas the mutant consisting of amino acids 210–398 of JLP was not co-immunoprecipitated with N-cadherin.

p38 MAPK inhibitor (SB203580) attenuated ADH-1-induced neurotoxicity (Fig. 2*C*), indicating an important role of p38 MAPK in the cell death caused by the inhibition of N-cadherin-based cell adhesion. However, because the application of SB203580 did not block ADH-1-induced cell death completely, we assume that other alterations in cellular signaling

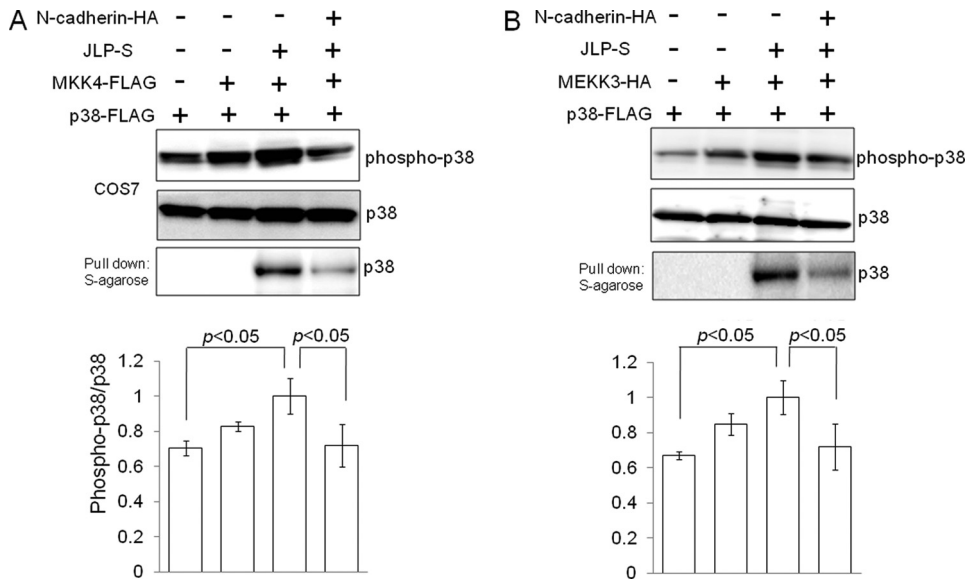

FIGURE 6. **N-cadherin expression inhibited JLP-associated p38 MAPK pathway.** *A*, COS7 cells were co-transfected with FLAG-tagged p38α, FLAG-tagged MKK4, S-tagged JLP, and HA-tagged N-cadherin. 24 h after transfection, the lysates were precipitated with S-protein-agarose. The lysates and precipitates were subjected to Western blot analysis with anti-phospho-p38 or p38 MAPK antibodies. One representative immunoblot result is shown. The band densities of phospho-p38 and p38 MAPK were quantified by National Institutes of Health Image. The phospho/total p38 MAPK ratio was calculated and analyzed by one-way ANOVA. N-cadherin expression significantly inhibited JLP/MKK4-mediated phosphorylation of p38 MAPK compared with that without expression of N-cadherin ($n = 3$, $p < 0.05$). Pull-down assay using S-protein-agarose showed that N-cadherin expression decreased the interaction between JLP and p38 MAPK. *B*, COS7 cells were co-transfected with FLAG-tagged p38α, HA-tagged MEKK3, S-tagged JLP, and HA-tagged N-cadherin. 24 h after transfection, the lysates were subjected to Western blot analysis with anti-phospho-p38 or p38 MAPK antibodies. One representative immunoblot result is shown. The band densities of phospho-p38 and p38 were quantified by National Institutes of Health Image. The ratio of phospho/total p38 MAPK was calculated and analyzed by one-way ANOVA. N-cadherin expression inhibited JLP/MEKK3-mediated phosphorylation of p38 MAPK significantly compared with that without expression of N-cadherin ($n = 3$, $p < 0.05$). Pull-down assay using S-protein-agarose showed that N-cadherin expression inhibited the interaction between JLP and p38 MAPK.

might be involved in neuronal death in addition to activation of p38 MAPK signaling. Notably, previous studies have shown that N-cadherin-mediated cell adhesion leads to the recruitment of PI3K into the N-cadherin adhesion complex followed by activation of Akt, which is an important regulator of anti-apoptotic pathways (12). Thus, down-regulation of PI3K/Akt signaling could also be participating in the ADH-1-induced cell death. Collectively, these results suggest that N-cadherin-mediated synaptic contact might contribute to neuroprotective signaling. Disruption of this synaptic contact might lead to neuronal cell apoptosis by perturbing these signalings.

Concerning a potential molecular link between N-cadherin and p38 MAPK signaling, we identified JLP as a novel N-cadherin-interacting protein in human brains by proteomic analysis. JLP is a member of the JIP (JNK-interacting protein) family, which provides a scaffolding function for the JNK/p38 MAPK signaling module. JLP is encoded by the *JIP4* gene, which generates three distinct splice variants, namely, JLP, JIP4, and SPAG9 (32). Specifically, JLP was identified as a scaffold protein involved in the p38 MAPK signaling pathway to bring p38 MAPK together with its upstream kinases (21). We also demonstrated that both the leucine zipper II domain and the region containing amino acids 160–209 of JLP were required for the association with N-cadherin, the same region for the interaction with p38 MAPK, which may explain the competitive regulation by N-cadherin. Moreover, our data showed that N-cadherin overexpression interfered with the physical interaction between JLP and p38 MAPK, thereby inhibiting the JLP-mediated activation of p38 MAPK signaling. Thus, it is plausible that perturbation of N-cadherin-based cell adhesion and/or reduced expression of N-cadherin could lead to aberrant activation of p38 MAPK via facilitating JLP-mediated p38 MAPK signaling.

Several recent reports have shown that cysteine dioxygenase type I associates with both N-cadherin and JLP in myoblasts (33, 34). However, our observation demonstrated an opposite result to the recent report showing that N-cadherin ligation activates p38 MAPK in a cysteine dioxygenase type I- and JLP-dependent manner during myoblast differentiation (35). Although it was unclear whether cysteine dioxygenase type I is involved in the p38 MAPK activation induced by the inhibition of N-cadherin-based cell adhesion in our study, we speculate that the discrepancy between our study and previous reports could be attributed to the differences in cell type specificity and the cellular context examined (*i.e.* myoblast differentiation *versus* neuronal degeneration). Variable roles of JLP and N-cadherin according to the differential cellular context have been demonstrated in previous studies. For example, JLP has been reported to negatively regulate NGF-induced neurite outgrowth via JNK inhibition during neuronal differentiation (36) as opposed to the previous report demonstrating that N-cadherin is involved in NGF-induced neurite outgrowth (37). Thus, the association between N-cadherin and JLP may be involved in differential cell fate by modulating signaling pathways including p38 MAPK and JNK according to the cellular context.

With respect to AD pathophysiology, the effect of Aβ on N-cadherin expression has been unclear. In this study, we

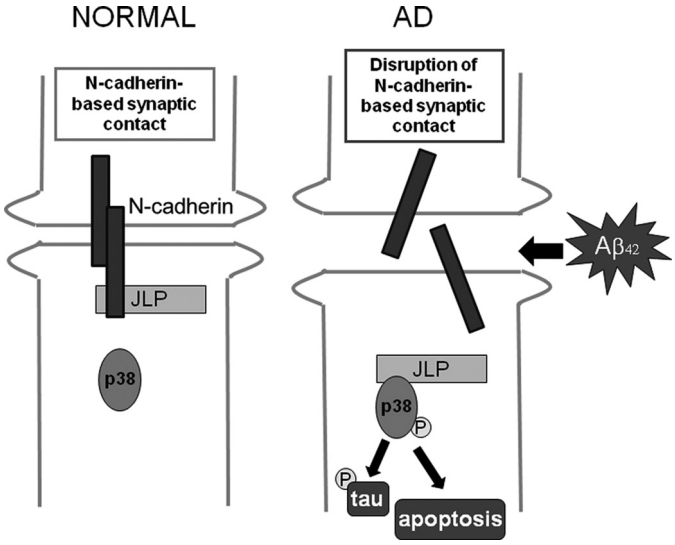

FIGURE 7. **Hypothetical model of AD pathogenesis caused by the disruption of N-cadherin-based synaptic contact.** *Left panel*, in the basal state at the synapse, N-cadherin-based synaptic adhesion stabilizes JLP, which suppresses neurotoxic p38 MAPK activation. *Right panel*, in AD, when N-cadherin-based synaptic contact is disrupted by $A\beta_{42}$, $A\beta_{42}$ could decrease N-cadherin expressions resulting in aberrant p38 MAPK activation followed by the subsequent Tau phosphorylation and neuronal death.

demonstrated that treatment with $A\beta_{42}$ decreased endogenous N-cadherin expression in murine primary neurons, whereas the aberrant phosphorylation of both p38 MAPK and p38 MAPK-sensitive Tau Ser-202/Thr-205 epitopes were simultaneously observed, as previously reported (29, 38). This result raises the possibility that $A\beta_{42}$ could interfere with N-cadherin-mediated synaptic contact, resulting in p38 MAPK activation. Recently, converging lines of evidence suggest that natural soluble $A\beta$ oligomers trigger synaptic loss (3, 20). Therefore, it is plausible that synaptic dissociation caused by $A\beta$ activates the p38 MAPK signaling pathway, leading to neuronal death as well as Tau phosphorylation. Interestingly, it has been shown that electroconvulsive and other excitatory stimuli induce arcadlin, a protocadherin, to promote activation of p38 MAPK and the endocytosis of N-cadherin at the synapse (39). Indeed, the neurodegeneration caused by $A\beta$ has long been related to the excessive activation of glutamate receptors, namely, excitotoxicity (30). Moreover, it is demonstrated that even physiological levels of $A\beta$ can enhance glutamate excitotoxicity (31). Importantly, we showed that $A\beta_{42}$-induced reduction of N-cadherin levels was NMDA receptor-dependent (Fig. 3*B*). Thus, it is possible that $A\beta$ enhances excitotoxity to promote the endocytosis of N-cadherin at the synapse, presumably followed by its degeneration by endosome/lysosome pathway. Alternatively, another group previously reported calpain-mediated degradation of N-cadherin after NMDA receptor stimulation (40). In either scenario, a decrease in N-cadherin expression could accelerate the JLP-mediated p38 MAPK activation, resulting in synaptic loss, increased phosphorylated Tau, and neuronal death in AD pathology. Overall, we suggest the possibility that JLP is a key molecule linking synaptic adhesion to p38 MAPK signaling involved in Tau phosphorylation and neuronal death associated with AD (Fig. 7).

In summary, our study suggested the physical and functional association between N-cadherin and p38 MAPK via JLP. We also demonstrated that perturbation of N-cadherin-mediated synaptic contact activated p38 MAPK pathway and increased Tau phosphorylation, leading to neuronal death. From observations obtained from the present study, we would like to extend our view of JLP function further and suggest that JLP is involved in the N-cadherin/p38 MAPK signaling pathway from synaptic adhesion to neurodegeneration. Because there were no reports focusing on the effect of $A\beta$ on synaptic adhesion, this is the first report proposing an attractive possibility that $A\beta_{42}$ may dissociate N-cadherin-mediated synaptic contact to trigger p38 MAPK activation, followed by neurodegeneration such as synaptic loss, Tau phosphorylation, and neuronal death in AD. Future study in this field could lead to a better understanding of AD pathophysiology.

Acknowledgments—We greatly thank Dr. E. P. Reddy (Temple University) for WT-JLP-S and its mutant derivatives, Dr. Johnson (National Jewish Center for Immunology and Respiratory Medicine) for MEKK3-HA, and Dr. Gupta (Adherex Technologies Inc.) for ADH-1.

REFERENCES

1. Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991) *Ann. Neurol.* **30,** 572–580
2. Selkoe, D. J. (2002) *Science* **298,** 789–791
3. Shankar, G. M., Bloodgood, B. L., Townsend, M., Walsh, D. M., Selkoe, D. J., and Sabatini, B. L. (2007) *J. Neurosci.* **27,** 2866–2875
4. Wolfe, M. S., De Los Angeles, J., Miller, D. D., Xia, W., and Selkoe, D. J. (1999) *Biochemistry* **38,** 11223–11230
5. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) *Nature* **398,** 513–517
6. Rogaev, E. I., Sherrington, R., Rogaeva, E. A., Levesque, G., Ikeda, M., Liang, Y., Chi, H., Lin, C., Holman, K., Tsuda, T., Mar, L., Sorbi, S., Nacmias, B., Piacentini, S., Amaducci, L., Chumakov, I., Cohen, D., Lannfelt, L., Fraser, P. E., Rommens, J. M., and St. George-Hyslop, P. H. (1995) *Nature* **376,** 775–778
7. Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J. F., Bruni, A. C., Montesi, M. P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R. J., Wasco, W., Da Silva, H. A., Haines, J. L., Perkicak-Vance, M. A., Tanzi, R. E., Roses, A. D., Fraser, P. E., Rommens, J. M., and St. George-Hyslop, P. H. (1995) *Nature* **375,** 754–760
8. Thinakaran, G. (1999) *J. Clin. Invest.* **104,** 1321–1327
9. Benson, D. L., and Tanaka, H. (1998) *J Neurosci.* **18,** 6892–6904
10. Murase, S., Mosser, E., and Schuman, E. M. (2002) *Neuron* **35,** 91–105
11. Togashi, H., Abe, K., Mizoguchi, A., Takaoka, K., Chisaka, O., and Takeichi, M. (2002) *Neuron* **35,** 77–89
12. Tran, N. L., Adams, D. G., Vaillancourt, R. R., and Heimark, R. L. (2002) *J. Biol. Chem.* **277,** 32905–32914
13. Baki, L., Shioi, J., Wen, P., Shao, Z., Schwarzman, A., Gama-Sosa, M., Neve, R., and Robakis, N. K. (2004) *EMBO J.* **23,** 2586–2596
14. Uemura, K., Kuzuya, A., Shimozono, Y., Aoyagi, N., Ando, K., Shimohama, S., and Kinoshita, A. (2007) *J. Biol. Chem.* **282,** 15823–15832
15. Uemura, K., Lill, C. M., Banks, M., Asada, M., Aoyagi, N., Ando, K., Kubota, M., Kihara, T., Nishimoto, T., Sugimoto, H., Takahashi, R., Hyman, B. T., Shimohama, S., Berezovska, O., and Kinoshita, A. (2009) *J Neurochem.* **108,** 350–360
16. Johnson, G. V., and Bailey, C. D. (2003) *Exp. Neurol.* **183,** 263–268
17. Hensley, K., Floyd, R. A., Zheng, N. Y., Nael, R., Robinson, K. A., Nguyen, X., Pye, Q. N., Stewart, C. A., Geddes, J., Markesbery, W. R.,

Patel, E., Johnson, G. V., and Bing, G. (1999) *J. Neurochem.* **72,** 2053–2058
18. Zhu, X., Rottkamp, C. A., Hartzler, A., Sun, Z., Takeda, A., Boux, H., Shimohama, S., Perry, G., and Smith, M. A. (2001) *J. Neurochem.* **79,** 311–318
19. Savage, M. J., Lin, Y. G., Ciallella, J. R., Flood, D. G., and Scott, R. W. (2002) *J. Neurosci.* **22,** 3376–3385
20. Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., and Malinow, R. (2006) *Neuron* **52,** 831–843
21. Lee, C. M., Onésime, D., Reddy, C. D., Dhanasekaran, N., and Reddy, E. P. (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99,** 14189–14194
22. Ito, M., Yoshioka, K., Akechi, M., Yamashita, S., Takamatsu, N., Sugiyama, K., Hibi, M., Nakabeppu, Y., Shiba, T., and Yamamoto, K. I. (1999) *Mol. Cell. Biol.* **19,** 7539–7548
23. Blank, J. L., Gerwins, P., Elliott, E. M., Sather, S., and Johnson, G. L. (1996) *J. Biol. Chem.* **271,** 5361–5368
24. Masliah, E., Terry, R. D., DeTeresa, R. M., and Hansen, L. A. (1989) *Neurosci. Lett.* **103,** 234–239
25. Gylys, K. H., Fein, J. A., Yang, F., Wiley, D. J., Miller, C. A., and Cole, G. M. (2004) *Am. J. Pathol.* **165,** 1809–1817
26. Williams, E., Williams, G., Gour, B. J., Blaschuk, O. W., and Doherty, P. (2000) *J. Biol. Chem.* **275,** 4007–4012
27. Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., Nairn, A. C., Salter, M. W., Lombroso, P. J., Gouras, G. K., and Greengard, P. (2005) *Nat. Neurosci.* **8,** 1051–1058
28. Zhu, X., Mei, M., Lee, H. G., Wang, Y., Han, J., Perry, G., and Smith, M. A. (2005) *Neurochem. Res.* **30,** 791–796
29. Reynolds, C. H., Nebreda, A. R., Gibb, G. M., Utton, M. A., and Anderton, B. H. (1997) *J. Neurochem.* **69,** 191–198
30. Hynd, M. R., Scott, H. L., and Dodd, P. R. (2004) *Neurochem. Int.* **45,** 583–595
31. Kihara, T., Shimohama, S., Sawada, H., Honda, K., Nakamizo, T., Shibasaki, H., Kume, T., and Akaike, A. (2001) *J. Biol. Chem.* **276,** 13541–13546
32. Kelkar, N., Standen, C. L., and Davis, R. J. (2005) *Mol. Cell. Biol.* **25,** 2733–2743
33. Kang, J. S., Feinleib, J. L., Knox, S., Ketteringham, M. A., and Krauss, R. S. (2003) *Proc. Natl. Acad. Sci. U.S.A.* **100,** 3989–3994
34. Takaesu, G., Kang, J. S., Bae, G. U., Yi, M. J., Lee, C. M., Reddy, E. P., and Krauss, R. S. (2006) *J. Cell Biol.* **175,** 383–388
35. Lu, M., and Krauss, R. S. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 4212–4217
36. Xu, H., Dhanasekaran, D. N., Lee, C. M., and Reddy, E. P. (2010) *J. Biol. Chem.* **285,** 3548–3553
37. Hansen, S. M., Berezin, V., and Bock, E. (2008) *Cell Mol. Life. Sci.* **65,** 3809–3821
38. Goedert, M., Jakes, R., and Vanmechelen, E. (1995) *Neurosci. Lett.* **189,** 167–169
39. Yasuda, S., Tanaka, H., Sugiura, H., Okamura, K., Sakaguchi, T., Tran, U., Takemiya, T., Mizoguchi, A., Yagita, Y., Sakurai, T., De Robertis, E. M., and Yamagata, K. (2007) *Neuron* **56,** 456–471
40. Jang, Y. N., Jung, Y. S., Lee, S. H., Moon, C. H., Kim, C. H., and Baik, E. J. (2009) *J Neurosci* **29,** 5974–5984